

## **LISTING OF CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Withdrawn) A composition comprising a polypeptide which comprises an amino acid sequence substantially identical to the sequence of SEQ ID NOs: 22-43, 59, or 73-84, or a fragment or variant thereof, in combination with a physiologically acceptable carrier.
2. (Withdrawn) A composition comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence substantially identical to the sequence of SEQ ID NOs: 22 - 43, 59, or 73-84, or a fragment or variant thereof, in combination with a physiologically acceptable carrier.
3. (Withdrawn) A composition comprising a nucleic acid molecule which comprises a nucleotide sequence substantially identical to the sequence of SEQ ID NOs: 1-21 or 60-72 or a fragment or variant thereof, in combination with a physiologically acceptable carrier.
4. (Withdrawn) A composition comprising a cell culture supernatant which comprises a polypeptide comprising an amino acid sequence substantially identical to the sequence of SEQ ID NOs: 22 - 43, 59, or 73-84, or a fragment or variant thereof, in combination with a physiologically acceptable carrier.
5. (Withdrawn) The composition of claim 4 wherein the polypeptide comprises 20% of the cell protein present in the composition.
6. (Withdrawn) The composition of any one of claims 1 through 5 further comprising a EspA, EspB, EspD, EspP, Tir, Shiga toxin 1, Shiga toxin 2, or intimin polypeptide.

7. (Withdrawn) The composition of any one of claims 1 through 6 further comprising an adjuvant.
8. (Withdrawn) A bacterium, or a preparation thereof, wherein the bacterium comprises a mutation in the bacterial genome in a nucleotide sequence that is substantially identical to SEQ ID NOs: 1-21 or 60-72.
9. (Withdrawn) A bacterium, or a preparation thereof, wherein the bacterium comprises a mutation in a gene substantially identical to *nleA*, *nleB*, *nleC*, *nleD*, *nleE*, *nleF*, *nleG* or *nleH* or a homologue thereof.
10. (Withdrawn) The bacterium of claim 8 or 9 wherein the bacterium is an A/E pathogen.
11. (Withdrawn) The bacterium of any one of claims 8 through 10 wherein the bacterium is enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), or *Citrobacter rodentium*.
12. (Withdrawn) The bacterium of claim 11 wherein the EHEC is EHEC O157:H7 or EHEC O157:NM.
13. (Withdrawn) The bacterium of claim 11 wherein the EPEC is EPEC O127:H6.
14. (Withdrawn) The bacterium of any one of claims 8 through 13 wherein the mutation attenuates virulence.
15. (Withdrawn) A composition comprising the bacterium of any one of claims 8 through 14, in combination with a physiologically acceptable carrier.

16. (Withdrawn) The composition of claim 15 wherein the bacterium is live.
17. (Withdrawn) The composition of claim 16 wherein the bacterium is administered orally.
18. (Withdrawn) The composition of claim 15 wherein the bacterium is killed.
19. (Withdrawn) The composition of claim 18 wherein the bacterium is administered parenterally.
20. (Withdrawn) The composition of any one of claims 15 through 19 further comprising an adjuvant.
21. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant is an oil-in-water emulsion or is a non-oil-in-water emulsion.
22. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises a mineral oil and dimethyldioctadecylammonium bromide.
23. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises one or more of an agent selected from the group consisting of an emulsifying agent, a muramyl dipeptide, an aqueous agent, a chitosan-based agent, a saponin, an oil, a lipopolysaccharide, a bacterial cell wall extract, a bacterial DNA, a bacterial complex, a synthetic oligonucleotide, and a aliphatic nitrogenous base.
24. (Withdrawn) The composition of claim 23 wherein the emulsifying agent is selected from the group consisting of one or more of a natural emulsifying agent, a synthetic

emulsifying agent, an anionic emulsifying agent, a cationic emulsifying agent, and a nonionic agent.

25. (Withdrawn) The composition of claim 24 wherein the natural emulsifying agent is selected from the group consisting of one or more of acacia, gelatin, lecithin, and cholesterol.

26. (Withdrawn) The composition of claim 24 wherein the anionic emulsifying agent is selected from one or more of the group consisting of a potassium salt of lauric acid, a potassium salt of oleic acid, a sodium salt of lauric acid, a sodium salt of oleic acid, an ammonium salt of lauric acid, an ammonium salt of oleic acid, a calcium salt of a fatty acid, a magnesium salt of a fatty acid, an aluminum salt of a fatty acid, a metallic soap, and an organic sulfonate.

27. (Withdrawn) The composition of claim 26 wherein the organic sulfonate is sodium lauryl sulfate.

28. (Withdrawn) The composition of claim 24 wherein the cationic emulsifying agent is cetyltrimethylammonium bromide.

29. (Withdrawn) The composition of claim 24 wherein the synthetic emulsifying agent is selected from the group consisting of one or more of a glyceryl ester, a polyoxyethylene glycol ester, a polyoxyethylene glycol ether, and a sorbitan fatty acid ester.

30. (Withdrawn) The composition of claim 28 wherein the glyceryl ester is glyceryl monostearate.

31. (Withdrawn) The composition of claim 28 wherein the sorbitan fatty acid ester is selected from one or more of the group consisting of a sorbitan monopalmitate and a polyoxyethylene derivative thereof.
32. (Withdrawn) The composition of claim 31 wherein the polyoxyethylene derivative is polyoxyethylene sorbitan monopalmitate.
33. (Withdrawn) The composition of claim 23 wherein the aqueous agent is aluminium hydroxide.
34. (Withdrawn) The composition of claim 23 wherein the oil is selected from the group consisting of one or more of a mineral oil, a vegetable oil, and an animal oil.
35. (Withdrawn) The composition of claim 34 wherein the vegetable oil is selected from the group consisting of one or more of canola oil, almond oil, cottonseed oil, corn oil, olive oil, peanut oil, safflower oil, sesame oil, and soybean oil.
36. (Withdrawn) The composition of claim 34 wherein the animal oil is selected from the group consisting of one or more of cod liver oil, halibut oil, menhaden oil, orange roughy oil and shark liver oil.
37. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises an oil component.
38. (Withdrawn) The composition of claim 37 wherein the oil component is selected from the group consisting of one or more of a single oil, and a mixture of oils.

39. (Withdrawn) The composition of claim 21 wherein the non-oil-in-water emulsion is selected from the group consisting of one or more of an oil emulsion, a water-in-oil emulsion, and a water-in-oil-in-water emulsion.
40. (Withdrawn) The composition of claim 21 wherein the oil-in-water emulsion is EMULSIGEN™ or EMULSIGEN PLUS™.
41. (Withdrawn) The composition of claim 23 wherein the oil is Amphigen®.
42. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises a Mycobacterial cell wall extract.
43. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises a Mycobacterial DNA.
44. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises a Mycobacterial cell wall complex.
45. (Withdrawn) The composition of claim 23 wherein the aliphatic nitrogenous base is selected from the group consisting of one or more of an amine, a quaternary ammonium compound, a guanidine, a benzamidine, and a thiuronium.
46. (Withdrawn) The composition of claim 23 wherein the aliphatic nitrogenous base is N,N-dioctadecyl-N,N-bis(2-hydroxyethyl)propanediarnine.
47. (Withdrawn) The composition of claim 7 or 20 wherein the adjuvant comprises dimethyl-dioctadecylammonium bromide.

48. (Withdrawn) The composition of any one of claims 21 through 47 wherein the adjuvant is present in the composition at a concentration of 20% to 40% (v/v).
49. (Withdrawn) A method of detecting the presence of an A/E pathogen in a sample, the method comprising:
- a) providing a sample; and
  - b) detecting the presence of a nucleic acid molecule comprising a nucleotide sequence substantially identical to a sequence selected from one or more of the group consisting of SEQ ID NOs: 1-21 or 60-72 or a fragment or variant thereof,
  - c) detecting the presence of a nucleic acid molecule encoding a polypeptide substantially identical to a sequence selected from one or more of the group consisting of SEQ ID NOs: 22-43, 59, 73-84 or a fragment or variant thereof, or
  - d) detecting the presence of a polypeptide comprising an amino acid sequence substantially identical to a sequence selected from one or more of the group consisting of SEQ ID NOs: 22-43, 59, 73-84 or a fragment or variant thereof,
- wherein the presence of the nucleic acid molecule or the polypeptide indicates the presence of an A/E pathogen in the sample.
50. (Withdrawn) The method of claim 49, wherein the sample is feces or blood.
51. (Withdrawn) The method of claim 49 or 50, wherein the detecting comprises contacting the nucleotide sequence with a probe or primer substantially identical to:
- a) a nucleotide sequence selected from the group consisting of one or more of SEQ ID NOs: 1- 21 or 60-72 or a fragment or variant thereof, or
  - b) a nucleotide sequence encoding a polypeptide substantially identical to one or more of SEQ ID NO: 22-43, 59, 73-84 or a fragment or variant thereof.
52. (Withdrawn) The method of claim 49 or 50, wherein the detecting comprises contacting the amino acid sequence with an antibody that specifically binds a sequence

selected from the group consisting of one or more of SEQ ID NOs: 22-43, 59, 73-84 or a fragment or variant thereof.

53. (Original) A method for eliciting an immune response against an A/E pathogen, or component thereof, in an animal comprising administering to the animal an effective amount of the composition of any one of claims 1-7 or 15-48, or comprising administering to the animal an effective amount of the bacterium of any one of claims 8-14, thereby eliciting an immune response in the animal.

54. (Original) A method for reducing colonization of an A/E pathogen in an animal, the method comprising administering to the animal an effective amount of the composition of any one of claims 1-7 or 15-48, or comprising administering to the animal an effective amount of the bacterium of any one of claims 8-14, hereby reducing colonization of the A/E pathogen in the animal.

55. (Original) A method for reducing shedding of an A/E pathogen in an animal comprising administering to the animal an effective amount of the composition of any one of claims 1-7 or 15-48, or comprising administering to the animal an effective amount of the bacterium of any one of claims 8-14, thereby reducing shedding of the A/E pathogen in the animal.

56. (Original) The method of any one of claims 53 through 55 wherein the animal is a ruminant.

57. (Original) The method of claim 56, wherein the ruminant is a bovine or ovine subject.



58. (Original) The method of any one of claims 53 through 55, wherein the animal is a human.

59. (Withdrawn) A method of treating or preventing infection by an A/E pathogen, the method comprising:

- a) identifying an animal having, or at risk for, an A/E pathogen infection; and
- b) administering to the animal an effective amount of a compound that attenuates the virulence of an A/E pathogen, wherein the compound inhibits the expression, secretion, or biological activity of a polypeptide comprising an amino acid sequence substantially identical to the sequence of any one of SEQ ID NOs: 22-43, 59, 73-84.

60. (Withdrawn) The method of claim 59, wherein the compound is an antisense nucleic acid molecule that is complementary to a nucleotide sequence substantially identical to the sequence of any one of SEQ ID NOs: 1-21, 59, 73-84.

61. (Withdrawn) The method of claim 59, wherein the compound is a siRNA.

62. (Withdrawn) A method of attenuating the virulence of an A/E pathogen, the method comprising mutating one or more of a gene selected from the group consisting of *nleA*, *nleB*, *nleC*, *nleD*, *nleE*, *nleF*, *nleG*, and *nleH*, or a homologue thereof in the A/E pathogen, or mutating one or more of a nucleotide sequence in the genome of the A/E pathogen, wherein the nucleotide sequence is selected from SEQ ID NOs: 1- 21 or 60-72, thereby attenuating virulence.

63. (Withdrawn) A method of screening for a compound that attenuates the virulence of an A/E pathogen, the method comprising:

- a) providing a system comprising:

- (i) a nucleic acid molecule comprising a nucleotide sequence substantially identical to SEQ ID NOs: 1-21 or 60-72 or a fragment or variant thereof; or
  - (ii) a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence substantially identical to SEQ ID NOs: 22-43, 59, 73-84 or a fragment or variant thereof; or
  - (iii) a polypeptide comprising an amino acid sequence substantially identical to SEQ ID NOs: 22-43, 59, 73-84 or a fragment or variant thereof;
- b) providing a test compound; and
- c) determining whether the test compound modulates the expression, secretion, or biological activity of the polypeptide or the nucleic acid molecule, wherein a change in the expression, secretion, or biological activity of the polypeptide or the nucleic acid molecule indicates a compound that attenuates the virulence of an A/E pathogen.

64. (Withdrawn) The method of claim 63 wherein the system is a cell.

65. (Withdrawn) The method of claim 63 or 64 wherein the cell is enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), or *Citrobacter rodentium*.

66. (Withdrawn) The method of claim 63 wherein the system is an in vitro system.

67. (Withdrawn) A method of producing a A/E pathogen polypeptide comprising:

a) providing a recombinant cell comprising:

(i) a nucleic acid molecule comprising a nucleotide sequence substantially identical to SEQ ID NOs: 1- 21 or 60-72; or

(ii) a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence substantially identical to SEQ ID NOs: 22-43, 59, or 73-84; and

b) growing the recombinant cell under conditions that permit expression of the polypeptide.

68. (Withdrawn) The method of claim 67 further comprising growing the recombinant cell under conditions that permit secretion of the polypeptide.

69. (Withdrawn) The method of claim 67 or 68 wherein the polypeptide is secreted from the cell.

70. (Withdrawn) The method of any one of claims 67 through 69 further comprising isolating the polypeptide.

71. (Original) The method of any one of claims 49 through 70 wherein the A/E pathogen is enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), or *Citrobacter rodentium*.

72. (Original) The method of claim 71 wherein the EHEC is EHEC O157:H7 or EHEC O157:NM.

73. (Original) The method of claim 71 wherein the EPEC is EPEC O127:H6.

74. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence substantially identical to the sequence of SEQ ID NOs: 22-43, 59, or 73-84.

75. (Withdrawn) An isolated nucleic acid molecule comprising a nucleotide sequence substantially identical to the sequence of SEQ ID NOs: 1-21 or 60-72.

76. (Withdrawn) A vector comprising the nucleotide sequence of claim 75.

77. (Withdrawn) The vector of claim 76 wherein the vector is capable of integrating into the genome of an A/E pathogen.

78. (Withdrawn) The vector of claim 76 wherein the vector is incapable of integrating into the genome of an A/E pathogen.
79. (Withdrawn) A host cell comprising the vector of any one of claims 76 through 78.
80. (Withdrawn) The host cell of claim 79 wherein the host cell is an A/E pathogen.
81. (Withdrawn) The host cell of claim 80 wherein the A/E pathogen is enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), or *Citrobacter rodentium*.
82. (Withdrawn) Use of the composition of any one of claims 1-7 or 15-48, the bacterium of any one of claims 8-14, the polypeptide of claim 74, or the nucleic acid molecule of claim 75, for the preparation of a medicament for eliciting an immune response against an A/E pathogen, or component thereof, or for reducing shedding or colonization of an A/E pathogen in an animal, or for treating or preventing infection by an A/E pathogen.
83. (Withdrawn) A kit comprising a reagent for detecting an A/E pathogen in a sample and a package insert with instructions for detecting the A/E pathogen in the sample.
84. (Withdrawn) The kit of claim 83, wherein the reagent comprises a probe or primer probe or primer substantially identical to:
- a) a nucleotide sequence selected from the group consisting of one or more of SEQ ID NOs: 1- 21 or 60-72 or a fragment or variant thereof, or
  - b) a nucleotide sequence encoding a polypeptide substantially identical to one or more of SEQ ID NO: 22-43, 59, 73-84 or a fragment or variant thereof.

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**PATENT**

85. (Withdrawn) The kit of claim 83, wherein the reagent comprises an antibody that specifically binds a sequence selected from the group consisting of one or more of SEQ ID NOs: 22-43, 59, 73-84 or a fragment or variant thereof.